

Research Article



Evaluation of Toxicological Potential of Methanolic Extract of *Chromolaena odorata* found in the Western Ghats of Indian Subcontinent Orally in Mice

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Abstract | Toxicological potential of *Chromolaena odorata*, a fast growing perennial weed was assessed in mice. Methanolic extract of *C. odorata* did not cause any mortality of mice. However, the oral feeding of extract resulted in sedation, loss of appetite, enteritis and decreased weight gain in treated mice. Significant elevation ($P < 0.05$) of the serum glutamic oxalo-acetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) was observed along with decrease in the serum superoxide dismutase (SOD) and catalase. No significant variation in the haematological parameters was observed in the treated mice except for the increase in counts of monocytes, lymphocytes as well as clotting and bleeding times. Gross pathological examination of extract treated mice revealed subcutaneous hemorrhages in different parts of the body, pale and friable liver, soft and congested lungs and kidneys. Histopathology of the liver revealed centrilobular necrosis with varying degrees of degenerative changes ranging from cloudy swelling to vacuolar changes of the periportal hepatic cells, congestion of the portal vein and proliferation of endothelial cells. Hence, the methanolic extract of invasive form of *C. odorata* found in the Wayanad region of Western Ghats of Indian subcontinent possesses severe toxic potential when given orally in mice.

Keywords | *C. odorata*, Enteritis, Centrilobular necrosis, Liver, Haemorrhages, Kidney, Lungs

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INTRODUCTION

Plants form the major source of nourishment for the livestock. Animals often feed on the highly palatable and highly nutritious fodder, however during times of scarcity, will be forced to feed on the less palatable, noxious or toxic weeds (Prasad et al., 2005). Kerala, the tiny southern Indian state has severe scarcity of cultivable land and hence fodder constraint has already broken the backbone of livestock industry. This will affect the production performance of the crossbred cattle of the state. *Chromolaena odorata* (L.) King and Robins formerly known as *Eupatorium odoratum* L. (family Asteraceae) is a fast growing perennial weed found mostly in farm lands (Aro et al., 2009). It is

commonly called as 'Communist Pacha' in Malayalam and has an even distribution throughout Kerala. Cattle occasionally graze on this plant due to the fodder scarcity.

The medicinal uses of the weed were largely exploited by traditional herbalists and were found useful in the management of burns, soft tissue wounds, skin infections (Phan et al., 2000; Phan et al., 2001; Panda and Ghosh, 2010), inflammation (Habtemariam, 2001; Owoyele et al., 2005; Ayyanar and Ignacimuthu, 2009; Hanh et al., 2011), malaria (Pisutthanan et al., 2005), diarrhea and diabetes (Odugbemi, 2006; Akinmoladun and Akinloye, 2007). In traditional medicine, decoction of the leaf was used as a cough remedy and as an ingredient with lemon grass and

guava leaves for the treatment of malaria and decoction of flowers was used as tonic, antipyretic and heart tonic (Vital and Rivera, 2009). In addition, anthelmintic and antimicrobial activities of the leaves of the plant were widely studied (Debashisha et al., 2010; Naidoo et al., 2011; Natheer et al., 2012). The fresh leaf was ground into a paste and applied topically on affected places to heal wounds (Kilani, 2006). The plant also possessed antibacterial, astringent, antioxidant, antipyretic and diuretic activities (Caceres et al., 1995; Gopinath et al., 2009). Besides, the hydroalcoholic extract of *C. odorata* was reported to possess hypoglycaemic activity and thereby reducing the cardiovascular risks (Ogbonnia et al., 2010). Additionally, the aqueous extract of leaves of *C. odorata* also protected against the development of atherosclerosis and coronary heart disease, as well as the dyslipidemic conditions that characterized obesity, hypertension and diabetes mellitus (Jude and Catherine, 2011). Furthermore, the immunopotentiating activities of the *C. odorata* leaf extract on the innate immunity of Balb/C mice were also reported (Leonora and Elena, 2012). The use of *C. odorata* in the formulation of diet for layers, monogastric animals and West African dwarf goats (Aro et al., 2009) were previously reported.

C. odorata extract owes its biological activities to the presence of a mixture of secondary plant products comprising of polyphenols with flavonoids as the major constituent followed by alkaloids. At least ten different flavonoids, including 3, 5, 4'-trihydroxy-7-methoxyflavone, 5, 7, 3'-trihydroxy-5-methoxyflavone and 3, 5, 7-trihydroxy-4'-methoxyflavone (Ling et al., 2007) were isolated and characterized.

Previous studies conducted in our own laboratory, confirmed the wound healing, anti-inflammatory as well as antimicrobial potentials of the plant leaf extracts (Unpublished). The dermal toxicity studies also confirmed that there was no cutaneous toxicity (Unpublished). However, the presence of sesquiterpene lactones and pyrrolizidine alkaloids in the plant was speculated as the cause of the acute toxicity resulting in death of cattle and goats in Karnataka (Prasad et al., 2005).

The increasing use of traditional medicines necessitates more scientific evidences of their harmlessness; hence there is an urgent need for systematic approach in evaluating their efficacy and safety profile. The secondary plant metabolites like other xenobiotics, are usually detoxified in the liver. In this process, they may damage the liver or form an active metabolite that will cause toxicity to other organs (Flanagan et al., 2007). Thus, the present study was undertaken to evaluate the toxic potential of the methanolic extract of *C. odorata* found in the Western Ghats of the Indian subcontinent orally in mice.

MATERIALS AND METHODS

PLANT MATERIAL

The fresh aerial parts of *Chromolaena odorata* were collected from the campus of College of Veterinary and Animal Sciences, Pookode, Wayanad district, Kerala. The plant was identified and authenticated by a botanist and a voucher specimen deposited at Calicut University Herbarium (CALI, Accession number: 6629), Calicut, Kerala for further reference.

CHEMICALS

All the chemicals, kits and solvents used in this study were obtained from M/s Merck India, Ltd (Bombay, India).

PREPARATION OF PLANT EXTRACT

The collected aerial parts of *C. odorata* were dried under shade and pulverized using a mechanical grinder. The powdered plant material (~2 kg) was extracted in methanol using a soxhlet extraction apparatus attached with rotary vacuum evaporator. The solvent was removed under reduced pressure (74 mbar) at 31°C using a rotary vacuum evaporator (Rotavac, Buchi, Switzerland) followed by drying at room temperature. The resulting dry methanolic extract was weighed. The per cent yield was calculated as 12.5 per cent (w/w). The extract was then stored in a refrigerator at 4°C. The appropriate dose of the dried extract was dissolved in distilled water and administered orally to animals limiting the final volume to 1mL.

ANIMALS

Twenty four male or female Balb/c mice weighing 20-25 g were obtained from the Small Animal Breeding Station, Pookode. All the animals were housed in an animal room under standard laboratory conditions of 21-22°C, light (12 h light/dark cycle) and humidity (50 ± 10 per cent). The animals were provided standard laboratory food and water *ad libitum*. They were acclimatized for 7 days prior to experiments. The experiments were performed according to the guiding principles in the use of animals in toxicology and approved by the Institutional Animal Ethics Committee.

PHYTOCHEMICAL EVALUATION OF THE CRUDE EXTRACT

Phytochemical analysis of the extract was performed on a qualitative basis and on TLC plate (Harborne, 1991).

EXPERIMENTAL DESIGN

The study was conducted for a period of 14 days. On the day of dosing, all animals were observed for mortality and clinical signs for the first 10 min, 30 min, 1, 2, 4 and 6 h after dosing. Thereafter, the animals were observed twice daily for mortality and clinical signs, for 14 days. The tests

were performed between 9 AM and 1 PM. Initial and final body weights of all animals were also recorded.

The mice were randomly divided into four groups each comprising of six animals. Group I animals served as control and received sterile distilled water orally for 14 days. Group II animals received carbon tetrachloride (CCl₄) at 0.5 mL/kg body weight in olive oil in 1:1 ratio as intraperitoneal injection (single day). Group III and IV animals received methanolic extract of *C. odorata* at the rate of 250 mg/Kg and 500 mg/Kg orally for 14 days. The body weights of these animals prior to and after the experiments were also noted.

Prior to sacrifice of treated animals, blood was withdrawn from the carotid artery with or without anticoagulant into sterile vials. Blood samples without anticoagulant were allowed to coagulate at 37°C for 30 minutes, refrigerated for 3hr followed by centrifugation at 5000 rpm for separation of serum. Serum samples were analyzed for SGOT and SGPT. Blood samples collected in vials containing disodium salt of ethylenediaminetetraacetic acid (EDTA sodium) at the rate of 1 mg/mL were used for the estimation of haematological parameters like total leukocyte count (TLC), total erythrocyte count (TEC), haemoglobin concentration (Hb), erythrocyte sedimentation rate (ESR), bleeding and clotting time and differential leucocyte count (DLC) (Schlam, 1975).

The animals of each group were sacrificed by anesthetic over dosage using ether. The liver and kidney were rapidly dissected, placed in ice cold normal saline for the estimation of specific enzymes like superoxide dismutase (Mimami and Yoshikawa, 1979) (SOD) and catalase (Cohen et al., 1970). The fresh tissues were analyzed immediately after collection. The tissues (1g) were homogenized using glass homogenizer with 9 mL of ice cold normal saline and were used for the enzyme estimation. The liver, kidney and lungs were promptly excised and fixed in 10 per cent formal saline for histopathological examination. The specimens were processed by standard procedure after embedding in paraffin wax. The blocks were sectioned at 5 µm thickness, stained using haematoxylin and eosin method (Smit and Burton, 1977) and examined microscopically.

STATISTICAL ANALYSIS OF DATA

Data were expressed as the mean ± S.E.M. (Standard Error of Mean). Statistical differences between control and treated groups were tested by one way ANOVA, followed by post-hoc analysis using standard SPSS software. For post hoc analysis Duncan's test was used. A value of P < 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

In the present study, the phytochemical analysis of the crude methanolic extract of *C. odorata* revealed the presence of alkaloids, steroids, glycosides, flavonoids, diterpenes, triterpenes and phenolic compounds. In general, secondary metabolites played protective roles (e.g. anti-oxidation, free radical scavenging, UV light-absorption, and anti-proliferation) and defended the plant against microorganisms such as bacteria, fungi and viruses. Many of these metabolites were bitter and / or toxic to potential herbivores, sometimes affecting even the herbivore's central and peripheral nervous systems (Rattan, 2010). The secondary metabolites were further subdivided into a number of distinct groups on the basis of their chemical structure and synthetic pathways. The most prevalent phytochemical groups being alkaloids, terpenes and phenolic compounds. Plant-derived alkaloids, by function and chemical nature, were generally toxic to mammals. Terpenes, on the other hand exhibited a wide range of effects within the CNS by their interactions with the noradrenergic system, cholinesterase inhibition and multiple direct interactions with the GABA system, including blockade of GABA-gated chloride channels and agonistic and antagonistic, direct and allosteric binding to GABA_A receptors (Rattan, 2010).

On gross evaluation of toxicity, no mortality was recorded until fourteen days in any of the groups. The animals of extract treated groups (group III and IV) exhibited sedation, 45 minutes post-treatment which persisted for 1.5 hours. An initial increase in appetite was observed in these animals which progressively decreased at the end of observation period. After the fifth day, the animals showed signs of enteritis and the whole perennial region of the extract treated animals were stained with faeces. By 14th day of the experiment all the animals were emaciated, totally exhausted and anorectic. The animals showed 'tucked up' abdomen and difficulty in breathing. The skin textures were lost and the hairs were curled in. Therefore, the toxicities observed in the present study were attributed to the presence of alkaloids and terpenes. Studies have further revealed that morbidity and mortality associated with exposure to terpenes were largely related to the degree of CNS depression. Terpenes being local irritants were also capable of causing GI signs and symptoms.

Assay of the biomarker enzymes revealed that the SGOT (AST) and SGPT (ALT) were significantly (P<0.05) elevated in all experimental groups compared to the control group (Figure 1). Whereas, there was a significant (P<0.05) decrease in the levels of antioxidant enzymes (SOD and catalase) (Figure 2 and 3) in the treated groups compared to control groups. However, no significant changes in the total erythrocyte and leucocyte counts as well as hemoglobin were observed in the treated animals compared to

Table 1: Effect of methanolic extract of *C. odorata* on total erythrocyte count, total leucocyte count and haemoglobin in mice

Group	TEC (million/mm ³)	TLC (thousands /mm ³)	Hb (g/dl)
I- Control	9.79 ± 1.09 ^a	10400 ± 110.12 ^a	11.8 ± 1.34 ^a
II- CCl ₄ @ 0.5 mL/kg	13.12 ± 0.93 ^b	8900 ± 100.12 ^b	12.4 ± 1.94 ^a
III – Methanolic extract @ 250 mg/ kg	13.79 ± 0.87 ^b	14600 ± 400.64 ^c	13.4 ± 3.38 ^a
IV- Methanolic extract @500 mg /kg	13.55 ± 0.77 ^b	8900 ± 110.11 ^b	13.6 ± 3.42 ^a

TEC = Total erythrocyte count; TLC = total leucocyte count; Hb = haemoglobin; CCl₄ = Carbon tetra chloride; n = 6; Values are Mean ± SEM, means bearing different superscripts a, b or c (P<0.05).

Table 2: Effect of methanolic extract of *C. odorata* on the differential leucocyte count in mice

Group	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophils (%)
I- Control	89.50 ± 1.05 ^a	10.50 ± 1.04 ^a	0	0
II- CCl ₄ @ 0.5 mL/kg	84.33 ± 2.59 ^a	15.67 ± 2.58 ^b	0	0
III – Methanolic extract @ 250 mg/ kg	81.50 ± 0.55 ^{ab}	16.0 ± 0.89 ^b	2.50 ± 0.84 ^a	0
IV- Methanolic extract @500 mg /kg	62.17 ± 1.6 ^b	31.33 ± 1.37 ^c	6.50 ± 1.0 ^b	0

CCL₄=Carbon tetra chloride; n = 6; Values are Mean ± SEM, means bearing different superscripts a, b or c (P<0.05).

the control (Table 1). The clotting time as well as bleeding time were long in treated group compared to control. Methanolic extract of *C. odorata* at concentration of 500 mg/Kg significantly (P<0.05) altered the counts of lymphocytes, neutrophils and monocytes in all treated groups compared to control (Table 2). The increase in the total leukocyte count was significant (P<0.05) in carbon tetra-chloride treated and group IV as well compared to control.

bloated with severe catarrhal enteritis. Foci of hemorrhage were observed in the intestinal epithelium. However, no subcutaneous hemorrhage was observed in carbon tetra-chloride treated group. The ballooning of the intestinal loops and catarrhal enteritis revealed the gastrointestinal toxic potential of the extract.

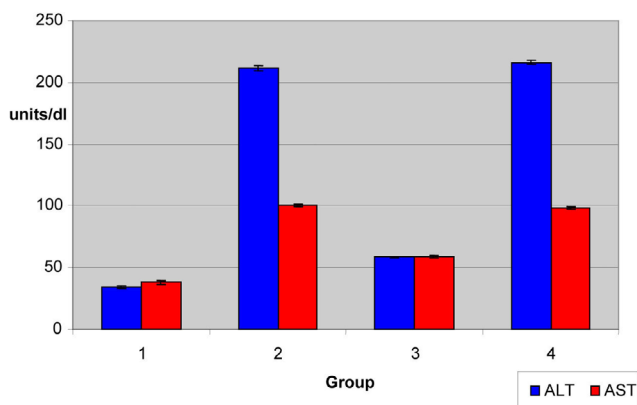


Figure 1: Effect of methanolic extract of *C.odorata* on serum AST and ALT in mice

Gross pathological examination of animals of extract treated group III and IV revealed subcutaneous hemorrhage in different parts of the body. The severity of changes were noted more in animals that received higher quantity of the extract. The liver was pale and friable with white spots on the surface. The lungs and the kidneys were congested and soft in consistency. The capsule was adhered to the kidney and was not peeling off easily. The intestinal loops were

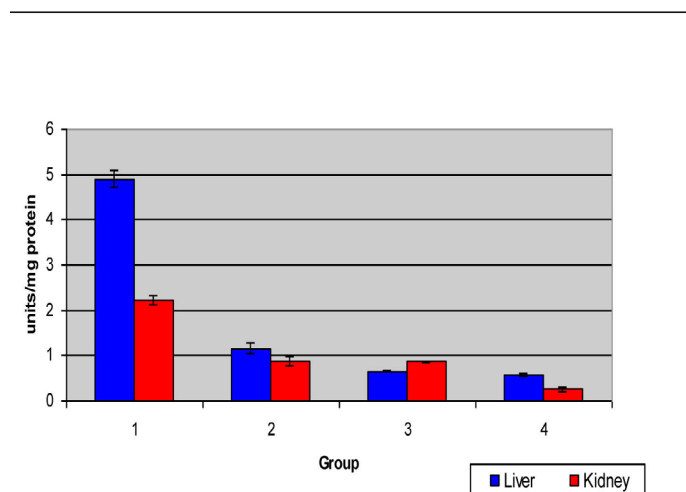


Figure 2: Effect of methanolic extract of *C.odorata* on tissue superoxide dismutase activity in mice

Histopathological examination of the liver sections further revealed centrilobular necrosis with varying degrees of degenerative changes ranging from cloudy swelling to vacuolar changes of the periportal hepatic cells, congestion of the portal vein and proliferation of endothelial cells in experimental groups. Varying degrees of necrosis were observed in hepatocytes. The degree of necrosis was severe in liver of group IV animals to an extent that the cellular architecture were completely lost (Figure 4). Section of kidney of methanolic extract treated animals revealed glomerular and tubular hemorrhages and interstitial cell

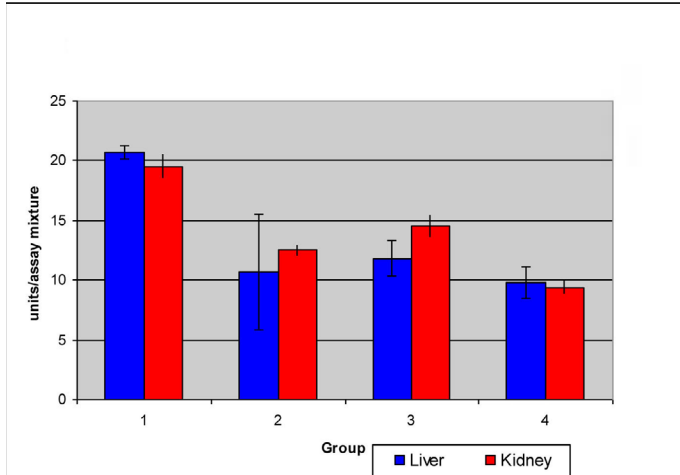


Figure 3: Effect of methanolic extract of *C.odorata* on tissue catalase activity in mice

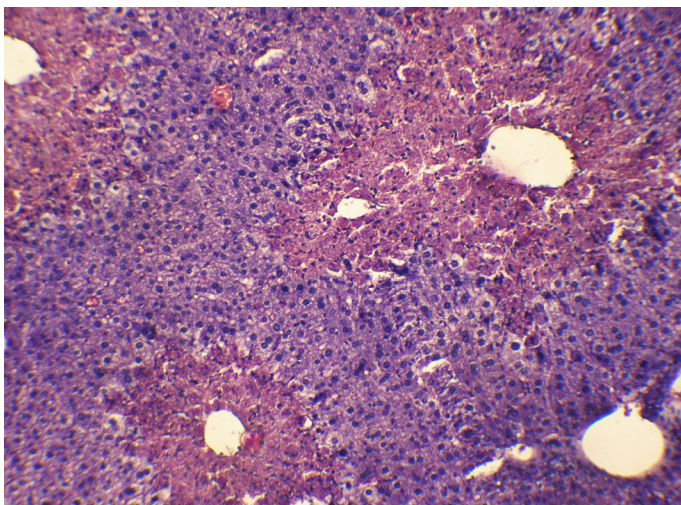


Figure 4: Section of liver of animals treated orally with methanolic extract of *C. odorata* (500 mg/ Kg) showing Kupffer cell proliferation and fatty degeneration (H&E 100X)

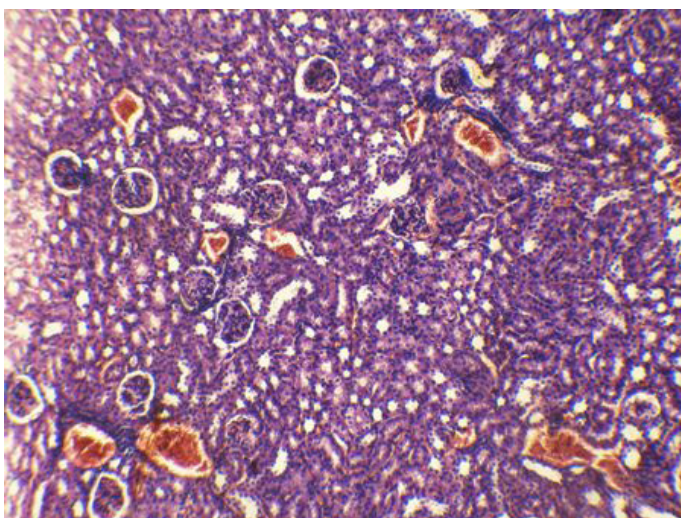


Figure 5: Section of Kidney of animals treated orally with methanolic extract of *C. odorata* (500 mg/ Kg) showing haemorrhages and interstitial cell proliferation (H&E 100X)

proliferation (Figure 5). Hemorrhages, rupture of alveolar septum, congestion of capillaries and proliferation of bronchiolar epithelial cells were observed in the lungs of extract treated animals (Figure 6). Histopathology of liver of carbon tetrachloride treated animals featured centrilobular necrosis whereas sections of kidney and lungs showed normal histoarchitecture. Previously, the sesquiterpene lactones and pyrrolizidine alkaloids of *C. odorata* were speculated as the cause of hepatotoxicity in cattle and goats in Karnataka (Prasad et al., 2005). In addition, the cytotoxic activity of sesquiterpene lactones isolated from the *Eupatorium* genus were also reported (Zhang et al., 2008). Moreover, the use of herbal teas made from pyrrolizidine alkaloid-producing plants to treat minor illnesses is also recognized as a cause of liver disease in parts of Africa and other tropical and subtropical countries (Roeder and Wiedenfeld, 2009).

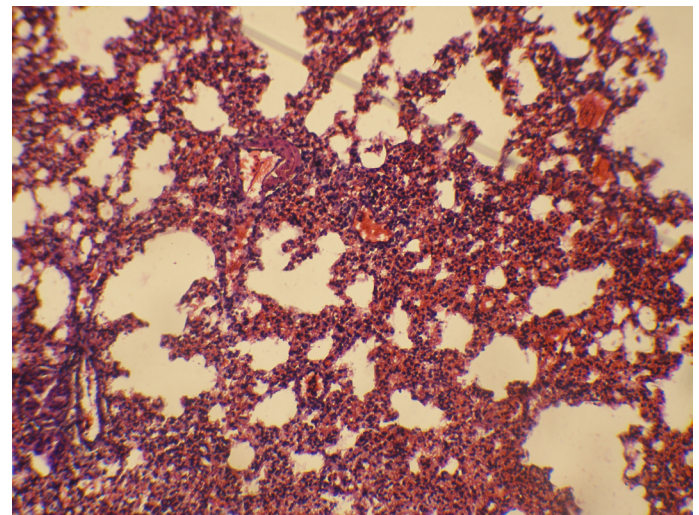


Figure 6: Section of lung of animals treated orally with methanolic extract of *C. odorata* (500 mg/Kg) showing haemorrhage, congestion of capillaries and proliferation of bronchiolar epithelial cells (H&E 100X)

The histopathological evaluation as well as the marker enzyme assays specifically provided an understanding of the extract’s potential to cause very high degree of hepatotoxicity. It is well known, that death of the hepatic cells leads to the rupture or damage of cell membranes, release of enzymes into the blood stream, thereby increasing the levels of marker enzymes in serum. In the kidneys also, tubular and glomerular hemorrhages were noticed. The damages seen in the liver were similar to that produced by carbon tetrachloride which point to the fact that the mechanism of action may be by oxidative damage. The decrease in the antioxidant enzymes like superoxide dismutase and catalase were also observed due to the oxidative stress. Carbon tetrachloride was a potent hepatotoxin which exerted its action mainly by production of free radicals especially trichloromethyl radicals which in turn bound covalently with the cellular proteins thereby damaging them. Since

the extract also produced a decrease in the antioxidant enzyme levels similar to that of carbon tetrachloride, it is presumed that the mechanism of toxicity might be similar. Further, the oxidative stress might also precipitate other underlying pathologies. Moreover, it has been reported that under such circumstances, even small amount of the plant in the diet itself could precipitate toxicity in an already compromised animal (Garg, 2002). Additionally, the terpenes are also reported to act through the oxidative pathway thereby indicating a mode of action through oxidative stress induced mechanism. Moreover, sesquiterpenes of genus *Eupatorium* induced cell death by alkylating and damaging the DNA (Ribeiro-Varandas et al., 2014). Further, the relative increase in the bleeding as well as clotting time indicated the relative decrease in the capacity of the liver for synthesizing the clotting factors due to cellular damage.

The results of the present study are highly contradictory to the studies conducted by Ogbonnia et al., 2010. They had reported an LD₅₀ value of 16.5 g/kg body weight in mice for the plant *C. odorata* that was abundant in Nigeria. Further, only mild toxic changes in liver and kidney were informed even at very high doses without any effect on the reproductive system. *C. odorata* collected from Nigeria was highly rich in saponins, moderately rich in phytates and tannins, with little content of alkaloids, flavonoids (aurone, chalcone, flavone and flavonol) and cyanogenic glycosides (Igboh et al., 2009). The difference observed in the toxicity of the same plant from two geographical regions might be due to the change in the constituents of the plant species. The plant grown in rain shade regions of the Western Ghats might have accumulated more toxins like nitrate and sesquiterpene lactones. Moreover, it has already been reported that the invasive forms of *C. odorata* found in Asia and West Africa and that occurring in Southern Africa differed from one another in morphology, biology and ecology. They were also functionally distinct entities which have been characterized as biotypes (Zachariades et al., 2009).

CONCLUSIONS

Chromolaena odorata, formerly known as *Eupatorium odoratum* is a fast growing perinneeal weed found mostly in farm lands. The present study was undertaken to evaluate the toxic potential of the methanolic extract of *C. odorata* found in the Western Ghats of the Indian subcontinent orally in mice. Based on the data presented herein, it may be concluded that the invasive form of *C. odorata* found in the Western Ghats of Indian subcontinent possessed severe toxic potential when given orally in mice. Further detailed investigation is needed to characterize the active agents responsible for the toxic and beneficial effects of the plant.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research paper.

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AUTHORS CONTRIBUTIONS

All authors contributed equally.

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